(54) Title: SUBCUTANEOUS ADMINISTRATION OF COAGULATION FACTOR VII

(57) Abstract: The invention relates to the use of a Factor VIIa for the manufacture of a medicament for treatment of a condition affectable by Factor VIIa, said medicament being for subcutaneous, intra-muscular or intradermal administration, and to the use of a Factor VIIa for the manufacture of a medicament for treatment of a condition affectable by Factor VIIa, wherein said medicament, when administered subcutaneously, intradermally or intramuscularly, shows a prolonged biological half-life.

SUBCUTANEOUS ADMINISTRATION OF COAGULATION FACTOR VII

FIELD OF INVENTION

The invention relates to the use of coagulation Factor VIIa for the manufacture of a
5   medicament for prevention or treatment of conditions affectable by Factor VIIa, wherein the
medicament is for subcutaneous, intramuscular or intradermal administration. The invention fur-
ther relates to the use of coagulation Factor VIIa for the manufacture of a medicament for
treatment of a condition affectable by Factor VIIa, wherein said medicament, when administered
subcutaneously, intradermally or intramuscularly, shows a prolonged biological half-life.

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BACKGROUND OF INVENTION

Blood coagulation is a process consisting of a complex interaction of various blood
components, or Factors, which eventually gives rise to a fibrin clot. Generally, the blood
components which participate in what has been referred to as the coagulation "cascade" are
15   proenzymes or zymogens, enzymatically inactive proteins which are converted to proteolytic
enzymes by the action of an activator, itself an activated clotting Factor. Coagulation Factors
that have undergone such a conversion are generally referred to as "active Factors", and are
designated by the addition of a lower case "a" suffix (e.g., Factor VIIa).

Activated Factor X ("Xa") is required to convert prothrombin to thrombin, which then
20   converts fibrinogen to fibrin as a final stage in forming a fibrin clot. There are two systems, or
pathways, that promote the activation of Factor X. The "intrinsic pathway" refers to those
reactions that lead to thrombin formation through utilisation of Factors present only in plasma.
A series of protease-mediated activations ultimately generates Factor IXa that, in conjunction
with Factor VIIIa, cleaves Factor X into Xa. An identical proteolysis is affected by Factor VIIa and
25   its co-Factor, tissue Factor, in the "extrinsic pathway" of blood coagulation. Tissue Factor is a
membrane bound protein and does not normally circulate in plasma. Upon vessel disruption,
however, it can complex with Factor VIIa to catalyse Factor X activation or Factor IX activation in
the presence of Ca^{++} and phospholipid. While the relative importance of the two coagulation
pathways in haemostasis is unclear, in recent years Factor VII and tissue Factor have been found
30   to play a pivotal role in the regulation of blood coagulation.

Factor VII is a trace plasma glycoprotein that circulates in blood as a single-chain
zymogen. The zymogen is catalytically inactive. Single-chain Factor VII may be converted to two-
chain Factor VIIa by Factor Xa, Factor XIIa, Factor IXa or thrombin _in vitro_. Factor Xa is believed
to be the major physiological activator of Factor VII. Like several other plasma proteins involved
35   in haemostasis, Factor VII is dependent on vitamin K for its activity, which is required for the γ-
carboxylation of multiple glutamic acid residues that are clustered in the amino terminus of the
protein. These γ-carboxylated glutamic acids are required for the metal-associated interaction of
Factor VII with phospholipids.

The conversion of zymogen Factor VII into the activated two-chain molecule occurs by cleavage of an internal peptide bond located approximately in the middle of the molecule. In human Factor VII, the activation cleavage site is at Arg_{152}-Ile_{153}. In the presence of tissue Factor, phospholipids and calcium ions, the two-chain Factor VIIa rapidly activates Factor X or Factor IX

5   by limited proteolysis.

Coagulation Factors are large proteins that are normally given intravenously to make the medicament directly available in the bloodstream. It would however be advantageous if a medicament could be given subcutaneously, intramuscularly or intradermally as these administration forms are much easier to handle for the patient, especially if the medicament

10   must be taken regularly during the whole life and treatment is to start early, e.g. when the patient is a child. However, a medicament with a very large and labile molecule normally has a low bioavailability if given subcutaneously, intramuscularly or intradermally, since the uptake is low and degradation is severe. Furthermore, such large proteins may be immunogenic when administered subcutaneously.

15   Recombinant human Factor VIIa (rFVIIa) is an activated coagulation Factor that is useful in the treatment of haemophiliacs that generate neutralising antibodies against Factor VIII or Factor IX. Factor VIII and Factor IX causes severe antibody formation in approximately 10% of the haemophilia patients. The action of rFVIIa (activation of the coagulation system via Factor X) is exerted in the vascular compartment of the body. The route of administration of rFVIIa has until

20   now been intravenously. As a result of the relatively short half-life, administration normally has to be repeated every 2.5 to 3 hours. An alternative form of administration which would result in a reasonable bioavailability and a long lasting absorption phase would allow an increase in dosing intervals and at the same time make self administration possible, thus increasing the convenience for the patient.

25   Factor VIIa is a glycoprotein with a molecular weight of approximately 50 kDa. It is therefore a sufficiently large molecule to point to the need for direct introduction into the bloodstream, since a very low bioavailability, if any, would be expected. Furthermore quite large doses may be required for an adult, for example during surgery. Consequently, Factor VIIa has conventionally been delivered intravenously to haemophilia A or B patients, either prophylacti-

30   cally or in response to bleeding episodes. Such repeated use of intravenous injections, while necessary to control the disease, may have side effects. Repeated injections may lead to the vein at the site of injection becoming fibrosed or occluded, a problem especially acute when treating the elderly. Also, when veins are small, as in babies, it may be difficult for the doctor to insert a needle into the vein to inject the required therapeutic dose.

35   The only coagulation Factor proteins that have been administered by subcutaneous injection are Factors VIII (170 - 300 kDa) and IX (60 kDa). These coagulation Factors are administered in the form of the single-chain zymogens, which have not yet been activated. These non-activated forms are more stable than the activated (cleaved) forms, which are degraded

much faster. Subcutaneous injection of these two proteins does not significantly change their pharmacokinetic properties (e.g., half life).

It has now been found that the activated, cleaved and thus more labile form of coagulation Factor VIIa can be delivered by subcutaneous, intramuscular or intradermal injection with sufficiently transport into the bloodstream in biologically active form and in adequate concentrations. It has also been found that FVIIa shows favourable pharmacokinetic properties (especially half life) when injected subcutaneously, intramuscularly or intradermally.

Factor VIIa is useful for administration to mammals, particularly humans, to control bleeding disorders, particularly bleeding disorders which are caused by clotting Factor deficiencies (haemophilia A and B), or clotting Factor inhibitors or bleeding disorders in patients not suffering from haemophilia A or B, for example, in patients suffering from von Willebrand's disease. Patients with von Willebrand's disease have a defective primary haemostasis because they lack or have an abnormal von Willebrand Factor protein. Bleeding disorders are also seen in patients with a normally functioning blood clotting cascade and may be caused by a defective platelet function, thrombocytopenia, or even by unknown reasons. Furthermore, FVIIa may be used for preventing or treating excessive bleedings in patients where the haemostatic system including the coagulation cascade and platelets is functioning normally. Such excessive bleedings are, for example, bleedings in association with tissue damage, for example surgery or trauma, especially in tissues rich in tissue Factor (TF). FVIIa may be used in such situations as well as when the bleeding is diffuse and poorly responding to current haemostatic techniques and therapies (e.g. haemorrhagic gastritis and profuse uterine bleeding). FVIIa may also be suitable for the treatment of bleedings occurring in organs with limited possibility for mechanical haemostasis such as brain, inner ear region, eyes as well as in association with the process of taking biopsies from various organs and in laparoscopic surgery.

Background art

International Patent Application No. WO 93/07890 relates to the treatment of haemophilia with FIX by subcutaneous injection.

International Patent Application No. WO 95/26750 relates to a formulation of FVIII or FIX suitable for subcutaneous injection for treatment of haemophilia A or B.

International Patent Application No. WO 95/28954 relates to concentrated preparations of FIX suitable for storage and subcutaneous injection.

LIST OF FIGURES

Fig. 1a-d shows the Individual Plasma Concentration Profiles of rFVIIa Following Intravenous (i.v.) and Subcutanous (s.c.) Administration of 0.2 mg/kg to Minipigs Nos. a-d;

Fig. 2a-d shows the Individual Plasma Activity Profiles of rFVIIa Following Intravenous (i.v.) and Subcutanous (s.c.) Administration of 0.2 mg/kg to Minipigs Nos. a-d;

Fig 3 shows the amino acid residue sequence of wild-type Factor VII.

LIST OF TABLES

Table 1 shows the PK-Results from ELISA-Assay following Dosing 0.2 mg/kg (0.33 ml/kg) rFVII i.v. and s.c. to Minipigs Weighing 11.4-13.4 kg.

Table 2 shows the PK-Results from Clot-Assay Following Dosing 0.2 mg/kg (0.33 ml/kg) rFVII i.v. and s.c. to Minipigs Weighing 11.4-13.4 kg.

SUMMARY OF THE INVENTION

It has now been found that activated coagulation Factor VIIa, (Factor VIIa or FVIIa), which is a very sensitive protein, can be administered subcutaneously, intramuscularly or intradermally, showing an acceptable absorption and a high level of active Factor VIIa protein in the blood. Furthermore, the plasma half life of both FVII antigen and FVII activity is increased significantly by the above administration and the t(max) is delayed by several hours.

Thus, in one aspect, the invention provides the use of a Factor VIIa for the manufacture of a medicament for treatment of a condition affectable by FVIIa, said medicament being for subcutaneous, intramuscular or intradermal administration

In another aspect, the invention provides the use of a Factor VIIa for the manufacture of a medicament for treatment of a condition affectable by FVIIa, wherein said medicament, when administered subcutaneously, intradermally or intramuscularly, shows a prolonged biological half-life.

In one embodiment, the invention provides the use of a Factor VIIa as the sole active coagulation factor for the manufacture of a medicament for treatment of a condition affectable by FVIIa, said medicament being for subcutaneous, intramuscular or intradermal administration

In another embodiment, the invention provides the use of a Factor VIIa as the sole active coagulation factor for the manufacture of a medicament for treatment of a condition affectable by FVIIa, wherein said medicament, when administered subcutaneously, intradermally or intramuscularly, shows a prolonged biological half-life.

In one embodiment of the invention, the Factor VIIa is recombinant human Factor VIIa. In another embodiment, the Factor VIIa is an amino acid sequence variant of Factor VIIa.

In one embodiment, the Factor VIIa variants having substantially the same or improved biological activity relative to wild-type Factor VIIa are those that exhibit at least about 25%, preferably at least about 50%, more preferably at least about 75% and most preferably at least about 90% of the specific activity of Factor VIIa that has been produced in the same cell type, when tested in one or more of a clotting assay, proteolysis assay, or TF binding assay as described in the present specification. In one embodiment, the Factor VIIa variants are selected from the list of [L305V]-FVIIa, [L305V/M306D/D309S]-FVIIa, [L305I]-FVIIa, [L305T]-FVIIa, [F374P]-FVIIa, [V158T/M298Q]-FVIIa, [V158D/E296V/M298Q]-FVIIa and [K337A]-FVIIa.

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In one embodiment, the medicament is for subcutaneous administration. In another embodiment, the medicament is for intramuscular administration. In another embodiment, the medicament is for intradermal administration.

In one embodiment, the medicament is in the form of a ready-to use aqueous solution. In another embodiment, the medicament is in the form of a lyophilised composition that prior to administration has been reconstituted in a pharmaceutically acceptable vehicle suitable for injection.

In one embodiment of the invention, the condition affectable by Factor VIIa is bleeding caused by lack of or defective coagulation Factors VIII, IX or VII, or by inhibitors against coagulation Factors VIII, IX or VII. In another embodiment, the condition is bleeding caused by a defective platelet function. In yet another embodiment, the condition is bleeding associated with excessive tissue damage or trauma.

The subject suffering from a condition affectable by Factor VIIa may be any animal, in particular a mammal - in a preferred embodiment, the mammal is a human being.

DETAILED DESCRIPTION OF THE INVENTION
Definitions

Factor VIIa, or FVIIa, may be purified from blood or produced by recombinant means. It is evident that the practice of the methods described herein is independent of how the purified Factor VIIa is derived and, therefore, the present invention is contemplated to cover use of any Factor VIIa preparation suitable for use herein.

As used herein, "Factor VIIa" encompasses wild-type Factor VII (i.e., a polypeptide having the amino acid sequence disclosed in U.S. Patent No. 4,784,950), as well as variants of Factor VII exhibiting substantially the same or improved biological activity relative to wild-type Factor VII. The term "Factor VIIa" is intended to encompass Factor VII polypeptides that have been proteolytically processed to yield their respective bioactive forms. Typically, Factor VII is cleaved between residues 152 and 153 to yield Factor VIIa.

The biological activity of Factor VIIa in blood clotting derives from its ability to (i) bind to tissue Factor (TF) and (ii) catalyze the proteolytic cleavage of Factor IX or Factor X to produce activated Factor IX or X (Factor IXa or Xa, respectively). For purposes of the invention, Factor VIIa biological activity may be quantified by measuring the ability of a preparation to promote blood clotting using Factor VII-deficient plasma and thromboplastin, as described, e.g., in U.S. Patent No. 5,997,864. In this assay, biological activity is expressed as the reduction in clotting time relative to a control sample and is converted to "Factor VII units" by comparison with a pooled human serum standard containing 1 unit/ml Factor VII activity. Alternatively, Factor VIIa biological activity may be quantified by (i) measuring the ability of Factor VIIa to produce of Factor Xa in a system comprising TF embedded in a lipid membrane and Factor X. (Persson et al., *J. Biol. Chem.* 272:19919-19924, 1997); (ii) measuring Factor X hydrolysis in an aqueous system; (iii) measuring

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its physical binding to TF using an instrument based on surface plasmon resonance (Persson, *FEBS Letts.* 413:359-363, 1997) and (iv) measuring hydrolysis of a synthetic substrate.

Factor VII variants having substantially the same or improved biological activity relative to wild-type Factor VIIa encompass those that exhibit at least about 25%, preferably at least about

5   50%, more preferably at least about 75% and most preferably at least about 90% of the specific activity of Factor VIIa that has been produced in the same cell type, when tested in one or more of a clotting assay, proteolysis assay, or TF binding assay as described above. Factor VII variants having a substantially modified biological activity relative to wild-type Factor VII include, without limitation, Factor VII variants that exhibit TF-independent Factor X proteolytic activity.

10   Variants of Factor VII, whether exhibiting substantially the same or better bioactivity than wild-type Factor VII, include, without limitation, polypeptides having an amino acid sequence that differs from the sequence of wild-type Factor VII by insertion, deletion, or substitution of one or more amino acids. Non-limiting examples of Factor VII variants having substantially the same biological activity as wild-type Factor VII include [S52A]-FVIIa, [S60A]-FVIIa (lino et al., *Arch.*

15   *Biochem. Biophys.* 352: 182-192, 1998); FVIIa variants exhibiting increased proteolytic stability as disclosed in U.S. Patent No. 5,580,560; Factor VIIa that has been proteolytically cleaved between residues 290 and 291 or between residues 315 and 316 (Mollerup et al., *Biotechnol. Bioeng.* 48:501-505, 1995); and oxidized forms of Factor VIIa (Kornfelt et al., *Arch. Biochem. Biophys.* 363:43-54, 1999). Non-limiting examples of Factor VII variants having substantially the same or

20   better biological activity compared to wild-type Factor VIIa include, but are not limited to, those described in Danish Patent Applications Nos. PA 2000 00734, PA 2000 01360, PA 2000 01361, and PA 2001 00477. Non-limiting examples include [L305V]-FVIIa, [L305V/M306D/D309S]-FVIIa, [L305I]-FVIIa, [L305T]-FVIIa, [F374P]-FVIIa, [V158T/M298Q]-FVIIa, [V158D/E296V/M298Q]-FVIIa and [K337A]-FVIIa.

25   In the present context the three-letter or one-letter indications of the amino acids have been used in their conventional meaning as indicated in table 1. Unless indicated explicitly, the amino acids mentioned herein are L-amino acids. It is to be understood, that the first letter in, for example, K337 represent the amino acid naturally present at the indicated position of FIG. 3, and that, for example, [K337A]-FVIIa designates the FVII-variant wherein the amino acid represented

30   by the one-letter code K naturally present in the indicated position in Fig. 3 is replaced by the amino acid represented by the one-letter code A.

Table 1: Abbreviations for amino acids:

Amino acid	Tree-letter code	One-letter code
Glycine	Gly	G
Proline	Pro	P
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Cysteine	Cys	C

Phenylalanine	Phe	F
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Histidine	His	H
Lysine	Lys	K
Arginine	Arg	R
Glutamine	Gln	Q
Asparagine	Asn	N
Glutamic Acid	Glu	E
Aspartic Acid	Asp	D

In this context, the term "a FVIIa unit" is defined by calibration against a secondary standard of the First International Standard 89/688 established in 1993. 50 international units (IU) human wild-type FVIIa correspond to about 1 µg protein.

5   In this context, the term "treatment" is designated to include prophylactic treatment of a FVIIa affectable condition.

Abbreviations

TF   tissue Factor

10   FVII   Factor VII in its single-chain, unactivated form

FVIIa   Factor VII in its activated form

rFVIIa   recombinant Factor VII in its activated form

FVIII   Factor VIII in its unactivated form

FIX   Factor IX in its unactivated form

15   IU   International Units

Preparation of compound

Human purified Factor VIIa suitable for use in the present invention is preferably made by DNA recombinant technology, e.g. as described by Hagen et al., Proc.Natl.Acad.Sci. USA 83: 2412-
20   2416, 1986 or as described in European Patent No. 200.421 (ZymoGenetics).

The Factor VII variants described herein may be produced by means of recombinant nucleic acid techniques. In general, a cloned wild-type Factor VII nucleic acid sequence is modified to encode the desired protein. This modified sequence is then inserted into an expression vector, which is in turn transformed or transfected into host cells. Higher eukaryotic cells, in particular cul-
25   tured mammalian cells, are preferred as host cells. The complete nucleotide and amino acid sequences for human Factor VII are known (see U.S. 4,784,950, where the cloning and expression of recombinant human Factor VII is described). The bovine Factor VII sequence is described in Takeya et al., J. Biol. Chem. 263:14868-14872 (1988)).

The amino acid sequence alterations may be accomplished by a variety of techniques.
30   Modification of the nucleic acid sequence may be by site-specific mutagenesis. Techniques for site-specific mutagenesis are well known in the art and are described in, for example, Zoller and Smith

(DNA 3:479-488, 1984) or "Splicing by extension overlap", Horton et al., Gene 77, 1989, pp. 61-68.
Thus, using the nucleotide and amino acid sequences of Factor VII, one may introduce the altera-
tion(s) of choice. Likewise, procedures for preparing a DNA construct using polymerase chain reac-
tion using specific primers are well known to persons skilled in the art (cf. PCR Protocols, 1990, Aca-
5   demic Press, San Diego, California, USA).

         Factor VII may also be produced by the methods described by Broze and Majerus,
J.Biol.Chem. 255 (4): 1242-1247, 1980 and Hedner and Kisiel, J.Clin.Invest. 71: 1836-1841, 1983. These
methods yield Factor VII without detectable amounts of other blood coagulation Factors. An even
further purified Factor VII preparation may be obtained by including an additional gel filtration as
10   the final purification step. Factor VII is then converted into activated FVIIa by known means, e.g. by
several different plasma proteins, such as Factor XIIa, IX a or Xa. Alternatively, as described by Bjo-
ern et al. (Research Disclosure, 269 September 1986, pp. 564-565), Factor VII may be activated by
passing it through an ion-exchange chromatography column, such as Mono Q® (Pharmacia fine
Chemicals) or the like.

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Pharmaceutical administration

         The regimen for any patient to be treated with FVIIa as mentioned herein should be de-
termined by those skilled in the art. The dose to be administered in therapy can be determined by a
physician and will depend on the route of administration (subcutaneous, intramuscular or in-
20   tradermal) and on the weight and the condition of the patient.

         Where FVIIa injected intravenously normally has to be administered every 2.5-3 hours,
FVIIa injected subcutaneously, intradermally or intramuscularly should be administered with an
interval of 12-48 hours, preferably 24 hours. FVIIa is preferably administered by subcutaneous in-
jections and in an amount of about 100-100,000 units per kg body weight, and preferably in an
25   amount of about 250 - 25,000 units per kg body weight corresponding to about 5-500 µg/kg.

Formulation of medicament

         An intravenous injection is normally 5-20 ml. It is normally preferred that an injection
given subcutanously is between 0.05 to 1 ml. The concentration of FVIIa must therefore be high
30   in such a medicament.

         The volume given can be more than 0.01 ml, suitable 0.1-2 ml, preferably 0.25-1.5 ml
and more preferable 0.5-1 ml.

         Additives increasing the bioavailability of FVIIa are suitably organic compounds per se,
salts thereof, emulsions or dispersions containing organic compounds per se or salts thereof, e.g.
35   dispersions of polar lipids, or any combination or sequence of addition thereof. Organic com-
pounds useful in the invention are e.g. amino acids, peptides, proteins, and polysaccharides. Pep-
tides include dipeptides, tripeptides, oligopeptides, such as collagen and gelatine. The collagen
and gelatine is preferably hydrolysed. Polysaccharides include e.g. chitosans, cyclodextrins, starch,

hyaluronic acids, dextrans, cellulose, and any derivatives, combinations and/or sequence of addi-
tion thereof. The starch is preferably hydrolysed. The emulsions include oil-in-water emulsions
with oil as the dispersed phase and water-in-oil dispersions with oil as the continuous phase. The
oil can be of vegetable or of animal origin or synthetically produced. Suitably, the vegetable oil

5   of the emulsions is soybean oil or safflower oil, or any combination thereof. Suitably the polar
liquids are one or more phospho- lipids or glycolipids or any combination thereof. The additives
increasing the bioavailability of FVIIa could be added to the formulation before drying or upon
reconstitution, or it could be added to a stable solution or dispersion containing FVIIa.

        Before administration, one or more aqueous solutions or dispersions could be added, in

10   any mixture or sequence, to the medicament of the present invention, which is a stable aqueous
solution, a dispersion or in dried form.

        The medicament could be in a dried form, preferably freeze-dried. Before administra-
tion, the dried product or composition can be reconstituted with an aqueous solution or a disper-
sion e.g. a suspension, a liposomal formulation or an emulsion.

15   The medicament can also be a stable aqueous solution ready for administration. It can
also be a dispersion, e.g. a suspension, a liposomal formulation or an emulsion.
The medicament is preferably given subcutanously. The FVIIa activity in the formulation is
preferably from about 0.1 mg/ml to about 50 mg/ml, more preferred from about 0.3 mg/ml to
about 25 mg/ml, more preferred from about 0.6 mg/ml to about 25 mg/ml, more preferred from

20   about 0.6 mg/ml to about 15 mg/ml, more preferred from about 1 mg/ml to about 15 mg/ml, and
even more preferred from about 3 mg/ml to about 15 mg/ml.

        The medicament may also comprise salt in order to give an isotonic solution, e.g. NaCl, KCl,
and/or it may comprise one or more other isotonicity establishing compounds, preferably in an
amount of more than 1.0 mg/ml.

25   Calcium, or other divalent metal ions, e.g. zinc, is necessary for the maintenance of the FVIIa
activity. It may be added as, for example, calcium chloride, but other salts such as calcium
gluconate, calcium glubionate or calcium gluceptate may also be used. The medicament
preferable comprises calcium chloride in an amount of more than 0.15 mg/ml.

        An amino acid is preferably used to buffer the system and it also protects the protein if

30   the formulation is freeze-dried. A suitable buffer could be glycine, lysine, arginine, histidine or
glycylglycine, preferred is glycylglycine..

        A non-ionic surfactant may also be present in the medicament. The surfactant is
preferable chosen from block-copolymers, such as a poloxamer, e.g. poloxamer 188, or a
polyoxyethylene sorbitan fatty acid ester, such as polyoxyethylene-(20)-sorbitan monolaurate or

35   polyoxyethylene-(20)-sorbitan monooleate. Preferred are polyoxyethylene-(20)-sorbitan
monooleate (Tween 20). Tween 20) is preferably used in a concentration of at least 0.01 mg/ml.
The non-ionic surfactant, if used, should preferably be present in an amount above the critical
micelle concentration (CMC). See Wan and Lee, Journal of Pharm Sci, 63, p. 136, 1974.

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Mono- or disaccharides (e.g. sucrose), polysaccharides such as low molecular weight dextrins, or sugar alcohols (e.g. sorbitol, glycerol or mannitol) may be added. The medicament may also comprise antioxidants such as bisulfite, ascorbate gluthathione, acetylcystein, tocopherol, methionin, EDTA, citric acid, butyl hydroxy toluene and /or butyl hydroxy anisole.

5   Complexing agents, such as EDTA and citric acid can also be present in small concentrations for stabilising the FVIIa molecules, if they exhibit a stronger affinity for destabilising metal ions than for calcium or other divalent metal ions, e.g. zn2+. Furthermore, preservatives such as benzyl alcohol, phenol, sorbic acid, parabens, and chlorocresol may be added.

The adjuvants are generally present in a concentration of from 0.001 to 4% w/v. The

10   pharmaceutical preparation may also contain protease inhibitors, e.g. aprotinin or tranexamic acid.

The pH of the preparation is preferably adjusted to a value in the interval of 2 - 9. Preparations having a pH from about 5.0 to about 7.5 are preferred, more preferred are preparations having a pH from about 5.0 to about 6.0, most preferred are preparations having a pH about 5.5.

15   Preferably, the used FVIIa is highly purified, i.e. has a specific activity of more than 40 IU/μg.

In one embodiment, the medicament consists of

rFVIIa	0.6 mg/ml (30,000 IU/ml)
Sodium chloride	2.92 mg/ml
Glycylglycine	1.32 mg/ml
Polysorbate 80	0.07 mg/ml
Calcium chloride, 2H2O	1.47 mg/ml
Mannitol	30.00 mg/ml
pH 5.5	

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25   (reconstituted with sterile water to 1 ml)

Conventional techniques for preparing pharmaceutical compositions, which can be used according to the present invention, are, for example, described in Remington: The Science and Practice of Pharmacy, 19th ed., 1995.

30   The medicaments may be sterilised by, for example, filtration through a bacteria-retaining filter, by incorporating sterilising agents into the medicaments, by irradiating the medicaments, or by heating the medicaments. They can also be manufactured in the form of sterile solid medicaments which can be dissolved in sterile water, or some other sterile injectable medium prior to or immediately before use.

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The present invention is further illustrated by the following examples. The presented examples are meant as an illustration of the invention, not as a limitation.

EXAMPLES

Materials and methods

The production of recombinant Factor VIIa (rFVIIa) was essentially performed as described in European Patent No. 200,421.

5 The FVIIa activity and the concentration were adjusted by dilution with water for injection and excipients were added in suitable amounts. The solution was then sterile filtered and freeze-dried.

Lyophilised powder of rFVIIa:

10 rFVIIa 0.6 mg/ml

Sodium chloride 2.92 mg/ml

Glycylglycine 1.32 mg/ml

Polysorbate 80 0.07 mg/ml

Calcium chloride, 2H2O 1.47 mg/ml

15 Mannitol   30.00 mg/ml

pH 5.5

Prior to use, the lyophilised composition was reconstituted in water to a total volume of 1.0 ml.

20

<u>Example 1</u>

<u>Animals</u>

The study was performed in 4 female Göttingen minipigs from Ellegaard Göttingen Minipigs ApS, Sorø Landevej 302, DK-4261, Dalmose, Denmark. At start of the acclimatisation pe-
5   riod the animals were 7 to 8 months old and the body weight was in the range 11.2 to 13 kg. A predosing period of one week (including an acclimatisation period of 5 days) was allowed before dosing.

Twice daily the animals were offered water and food (175 g Altromin 9023 for the first 2 days, thereafter 200 g).

10   The study was performed in a thermostated room at $21\pm3°C$.

<u>Drugs and chemicals</u>

rFVIIa was used for dosing. The substance was dissolved in sterile H_2O to give 0.6 mg/ml. All other chemicals were obtained from commercial sources and were of analytical grade.

<u>Experimental design</u>

15   The animals were dosed once intravenously (i.v.) and once subcutanously (s.c.) separated by a wash-out period of one week as follows:

Animal No.	Route of dosing	
	First dosing	Second dosing
1	s.c.	i.v.
2	s.c.	i.v.
3	i.v.	s.c.
4	i.v.	s.c.

The dose was 0.2 mg/kg body weight corresponding to 0.33 ml/kg body weight.
20   The i.v. dose was given via a needle or a short catheter in an ear vein. Immediately after dosing the needle/catheter was flushed with 2-5 ml sterile, isotonic water.

The s.c. dose was given behind the pinna. The area of the dosing was marked with a colour marker.

<u>Blood and tissue sampling</u>

25   Blood samples were collected via needle puncture of the jugular vein/bijugular trunk. In connection with i.v. dosing the samples were collected before, and 6, 15, 30, 60 and 90 minutes and 2, 3, 4, 6 and 8 hours after dosing. Following s.c. dosing the samples were collected at 30, 60 minutes and 2, 3, 4, 5, 6, 8, 10, 12 14 and 24 hours as well as before dosing.

All blood samples were taken within 1 minute from stipulated time except for two sam-
30   ples (animal no. 4, 14 and 24 hours after s.c. dosing) that were taken 2 minutes after scheduled time.

The blood samples (3 ml) were collected in Vacutainers containing citrate for stabilisa-
tion and kept in ice-water until centrifugation (10 min, +4°C, about 1268xG). Two aliquots each of 150 Tl were taken from each sample. To one of the aliquots 1350 Tl of the buffer used for

ELISA assay were added and the mixture devided between two Nunc Cryotubes labelled with "ELISA" appart from identification and stored at approximately -20°C pending transfer to the Immunochemistry deparment for assay. To the other aliquot 1350 µl of the buffer used for Clot assay were added and the mixture devided between two Nunc Cryotubes. The tubes were la-

5   belled with "CLOT" apart from identification and stored at -80°C pending transfer to the Immunochemistry department, Novo Nordisk, for assay. Buffer was added to the samples within 0.5 h after sampling and the samples were frozen within 1 h after sampling.

The day after the second dosing all animals were anaesthetized with an i.p. injection of mebumal and killed by exsanguination. The subcutaneous injection sites were located, examined

10 macroscopically and representative samples were removed and fixed in phosphate buffered neutral 4% formaldehyde and transferred to the Pathology department, Novo Nordisk, for histopatological examination.

<u>Analytical methods</u>

The concentration of rFVIIa was determined by an ELISA and the activity of rFVIIa by a Clot assay.

15 <u>ELISA</u>

The ELISA assay was FVII:Ag ELISA performed as described in Kit insert no. 1994.09/db version 1.0 (Danish version). The assay has previously been validated for human and rat plasma. A preliminary validation showed no indication of problems by using the assay for analysis in pig plasma.

20 The assay is a two-site monoclonal immunoenzymatic assay using peroxidase as the marker enzyme. The microtiter wells are precoated with a specific anti-Factor VII monoclonal antibody. Thereafter both sample and enzyme-labelled antibody are added to the well. During the following step, a "sandwich" is formed between the solid-phase antibody, the Factor VII molecule and the enzyme-labelled specific Factor VII monoclonal antibody. Following a washing step,

25 where unbound enzyme-labelled antibody is remove, the activity of the bound peroxidase is measured by enzyme's ability to transform a colourless substrate to a coloured product. The colour development is stopped by addition of sulphuric acid and is measured at 492 nm. As standard is used rFVIIa delivered with the assay. The calibration of the standard is based on absorbency measurement at 280 nm.

30 <u>Clot-assay</u>

The Clot-assay was FVIIa:Clot (Stago) performed according to the kit insert. The assay has previously been validated for human and rat plasma. A preliminary validation showed no indication of problems by using the assay for analysis in pig plasma.

The recombinant soluble tissue Factor (rsTF) possesses a coFactor function specific for

35 FVIIa. Consequently the FVII present in the test plasma does not interfere in the assay. The rsTF in the presence of FVIIa, phospholipids and Ca^{++} produces coagulation of plasma. The observed clotting time bears an inverse relationship with the FVIIa level initially present in plasma. As standard

is used rFVIIa delivered with the assay. The calibration of the standard is based on a comparison with the international FVIIa standard.

Analysis of data

Results from ELISA as well as from Clot assay were subjected to non-compartmental pharmacokinetic analysis using the PC-based software WinNonlin (Scientific Consulting Inc.).

Results and discussion

Results from the ELISA and Clot assays are given in Appendix A. Individual plasma concentration profiles are given in Figures 1 and 2. while individual pharmacokinetic parameters are given in Tables 1 and 2.

None of the predose samples had measurable concentrations or activities indicating sufficient wash-out between the two dosing periods.

The plasma concentration and activity profiles (Fig. 1-2) show an extended absorption phase following s.c. administration resulting in a t_{max} mean of 6.5 (range 5-8 hours) and 4.0 (range 1.0-10.0) for concentration and activity, respectively.

Table 1.

PK-Results from ELISA-Assay following Dosing 0.2 mg/kg (0.33 ml/kg) rFVII i.v. and s.c. to
Minipigs Weighing 11.4-13.4 kg

Ani-mal No.	C_{max} (ng/ml)		t_{max} (h)	$AUC_{0-\infty}$ (ng·h/ml)		Extrapol. AUC (%)		f (%)	Half-life (h)	
	i.v.	s.c.	s.c.	i.v.	s.c.	i.v.	s.c.	s.c.	i.v.	s.c.
1	2130	86.3	8.0	4980	1711	11.2	14.7	34.4	2.7	7.7
2	2447	245.0	8.0	6960	4195	14.7	12.1	60.0	3.3	6.5
3	2134	142.6	5.0	6414	1849	13.0	12.7	28.8	2.9	7.2
4	2200	233.8	5.0	6659	2903	16.4	5.4	43.6	3.5	4.8
Mean	2228	176.9	6.5	6261	2665	13.8	11.2	41.7	3.5[a]	6.4[a]
SD	150	75.9	1.7	886	1151	2.3	4.0	13.6		

[a] : Harmonic mean

Table 2.

PK-Results from Clot-Assay Following Dosing 0.2 mg/kg (0.33 ml/kg) rFVII i.v. and s.c. to
Minipigs Weighing 11.4-13.4 kg

Animal No.	C_{max} (mIU/ml)		t_{max} (h)	$AUC_{0-\infty}$ (mIU·h/ml)		Extrapol. AUC (%)		f (%)	Half-life (h)	
	i.v.	s.c.	s.c.	i.v.	s.c.	i.v.	s.c.	s.c.	i.v.	s.c.
1	161454	4609	2.0	201887	67038	7.0	11.4	33.2	2.6	6.9
2	183766	9155	10.0	274157	126227	4.9	5.0	46.0	2.3	4.7
3	156908	5415	3.0	240033	63730	6.7	12.0	26.6	2.6	7.0
4	149559	13355	1.0	249408	135294	12.4	8.1	54.2	3.6	6.4
Mean	162922	8134	4.0	241371	98073	7.8	9.1	40.0	2.7[a]	6.1[a]
SD	14735	4005	4.1	30001	37950	3.2	3.2	12.4		

[a] : Harmonic mean

Consequently, C_{max} values were greatly reduced compared to those following i.v. ad-
ministration (Tables 1 and 2). The AUCs following s.c. administration were reduced compared to
those following i.v. administration (Tables 1 and 2). However, the extent of bioavailability was
reasonably good as the mean f was 41.7% (range 28.8-60.0%) and 40.0% (range 26.6-54.2%) as
estimated from ELISA and Clot assay results, respectively.

The half-life following s.c. administration was for all animals and for concentration as
well as for activity results increased compared to that after i.v. administration (Tablea 1 and 2)

The reason for that is most likely the so called "flip-flop" meaning that the rate of absorption is more slow than the rate of elimination. The half-life following s.c. administration is therefore a measure of the absorption rate rather than of the elimination rate.

Conclusion

The bioavailability of rFVIIa following subcutaneous administration to minipigs was sufficiently high to make this route of administration interesting in man. The absorption phase following s.c. administration was prolonged to an extent that might allow significantly increased dosing intervals in humans compared to those needed in connection with i.v. administration.

Table 3.

rFVII Plasma Concentration and Activity Data

I.v. Adm. Time (h)	Results from ELISA (ng/ml)				Resluts from Clot assay (mIU/ml)			
	Pig 1	Pig 2	Pig 3	Pig 4	Pig 1	Pig 2	Pig 3	Pig 4
0	$<^a$	$<^a$	$<^a$	$<^a$	$<^b$	$<^b$	$<^b$	$<^b$
0.1	2129.9	2447.2	2134.1	2200	131950	169150	130260	125680
0.25	1713.9	2058.5	1921.7	1810.2	97488	149376	98528	96816
0.5	1389.5	1801.4	1699.7	1639.3	72332	106160	93384	81248
1	1151.5	1285.2	1336.5	1291.7	50155	67885	55860	50855
1.5	869.1	1157.9	1134.5	1167.8	36960	60625	59352	49193
2	669.4	1306.5	1023.2	1019.9	25011	36475	29230	33830
3	586.7	731.5	718.9	717.5	16349	29708	26200	26675
4	410.2	515.7	524.7	472.1	11149	13514	12923	12913
6	217.2	319.9	290.4	338.7	8641	7484	7812	9019
8	144.8	219.8	200.7	215.1	3813	4062	4364	5969
s.c. Adm. Time (h)								
0	$<^a$	$<^a$	$<^a$	$<^a$	$<^b$	$<^b$	$<^b$	$<^b$
0.5	$<^a$	45.9	$<^a$	68.6	3263	3889	1118	5904
1	53.5	84	$<^a$	171.7	3907	6293	1990	13355
2	74.2	159.1	54.3	185.1	4609	8568	3509	11943
3	79.2	156.8	97.5	202.6	4315	6744	5415	12707
4	77.1	184.1	104.2	178.6	3889	7020	4510	10743
5	67.3	192.3	142.6	233.8	3108	n.s.	3589	7720
6	71.6	224	$<^{a,c}$	178.6	2740	6663	1642	6591
8	86.3	245	109.8	122.7	2884	6927	3585	7000
10	79.8	225.8	91.9	140.4	3867	9155	3310	5630
12	58.4	193.3	69.9	118.6	2542	4983	2555	4700
14	75.6	161.9	60.5	106.6	2066	4543	1977	2970
24	22.5	54.4	22.5	22.5	764	929	759	1180

5   [a] : Below limit of quantitation (45 ng/ml)
       [b] : Below limit of quantitation (117.6 mIU/ml)
       [c] : Excluded as outliner
       n.s.: No sample

CLAIMS

1. Use of a Factor VIIa for the manufacture of a medicament for treatment of a condition affectable by Ffctor VIIa, said medicament being·for subcutaneous, intramuscular or intradermal administration

2. Use according to claim1, wherein Factor VIIa is recombinant human Factor VIIa.

3. Use according to claim 1, wherein Factor VIIa is an amino acid sequence variant of Factor VIIa.

4. Use according to any one of claims 1-3, wherein the medicament is for subcutaneous administration.

5. Use according to any one of claims 1-4, said medicament being a stable, ready-to-use aqueous solution.

6. Use according to any one of claims 1-4, said medicament being a lyophilised composition that is reconstituted with a pharmaceutical acceptable injection vehicle prior to administration.

7. Use of a Factor VIIa for the manufacture of a medicament for treatment of a condition affectable by Factor VIIa, wherein said medicament, when administered subcutaneously, intradermally or intramuscularly, shows a prolonged biological half-life.

8. Use according to claim 7, wherein Factor VIIa is recombinant human Factor VIIa.

9. Use according to claim 7, wherein Factor VIIa is an amino acid sequence variant of Factor VIIa.

10. Use according to any one of claims 7-9, wherein the medicament is for subcutaneous administration.

11. Use according to any one of claims 7-10, said medicament being a stable, ready-to-use aqueous solution.

12. Use according to any one of claims 7-10, said medicament being a lyophilised composition that is reconstituted with a pharmaceutical acceptable injection vehicle prior to administration

13. Use according to claim 1 or claim 7, wherein the condition is bleeding caused by lack of or defective coagulation Factors VIII, IX or VII, or inhibitors against coagulation Factors VIII, IX or VII.

14. Use according to claim 1 or claim 7, wherein the condition is bleeding caused by a defective platelet function.

15. Use according to claim 1 or claim 7, wherein the condition is bleeding associated with excessive tissue damage or trauma.

Figure 1. Individual Plasma Concentration Profiles of rFVIIa Following Intravenous (i.v.) and Subcutanous (s.c.) Administration of 0.2 mg/kg to Minipigs
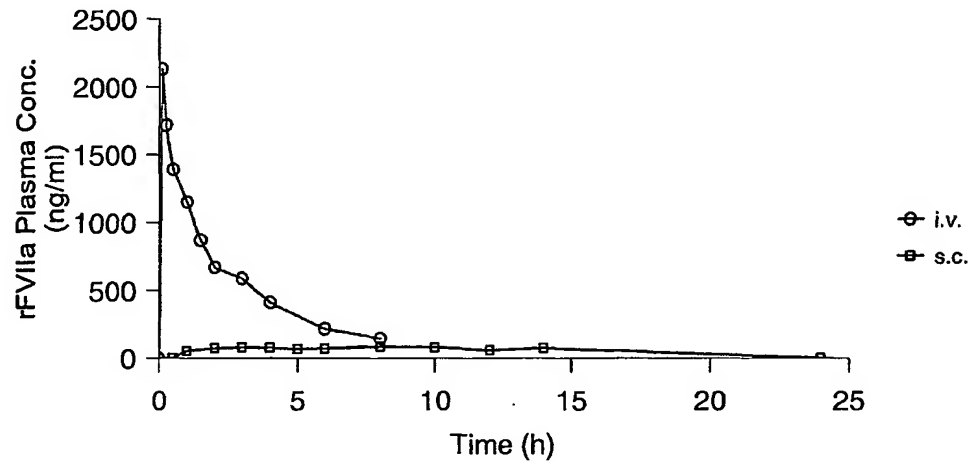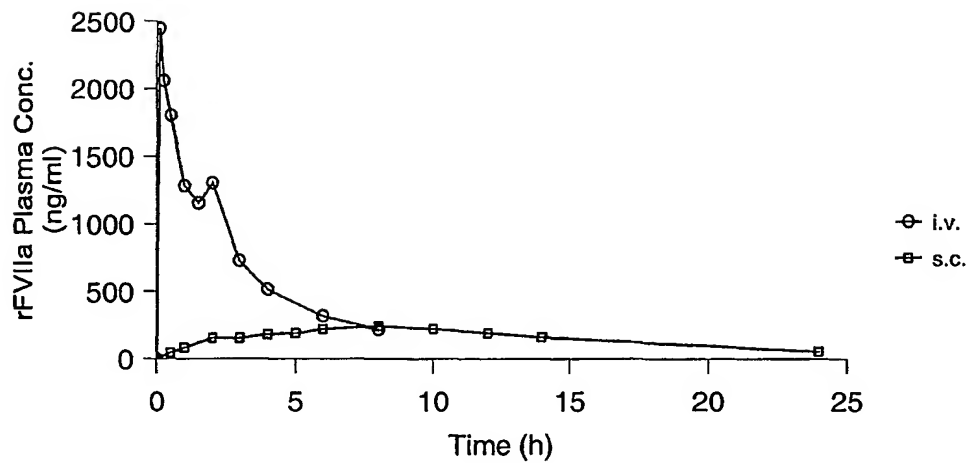
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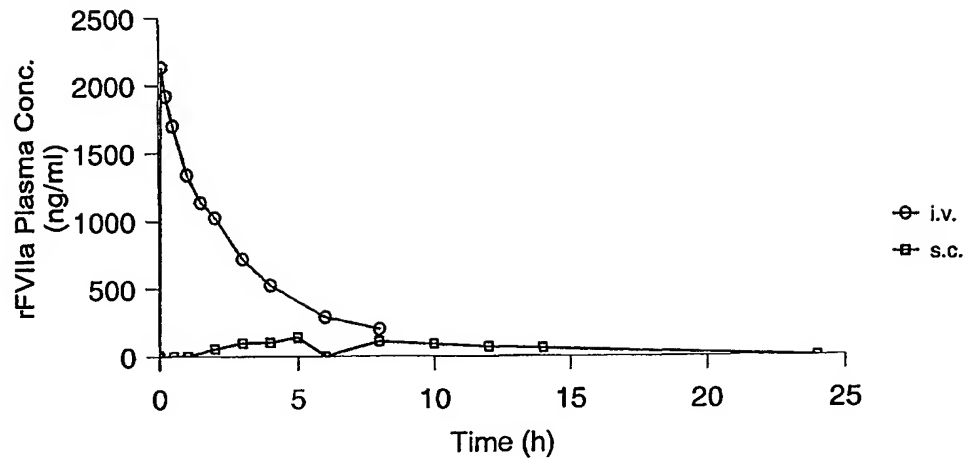

Fig. 1a

Animal_No. = 2

Fig. 1b

Animal_No. = 3

Fig. 1c

Animal_No. = 4

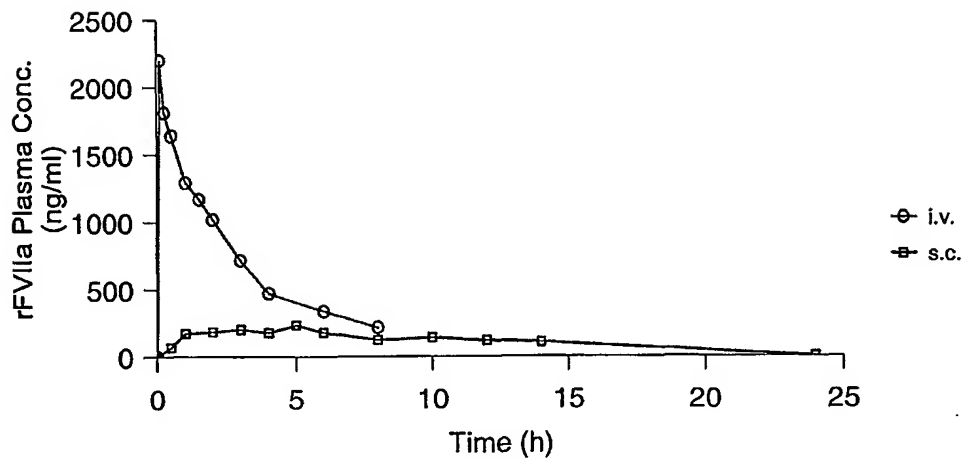Fig. 1d

Figure 2. Individual Plasma Activity Profiles of rFVIIa Following Intravenous (i.v.) and Subcutanous (s.c.) Administration of 0.2 mg/kg to Minipigs
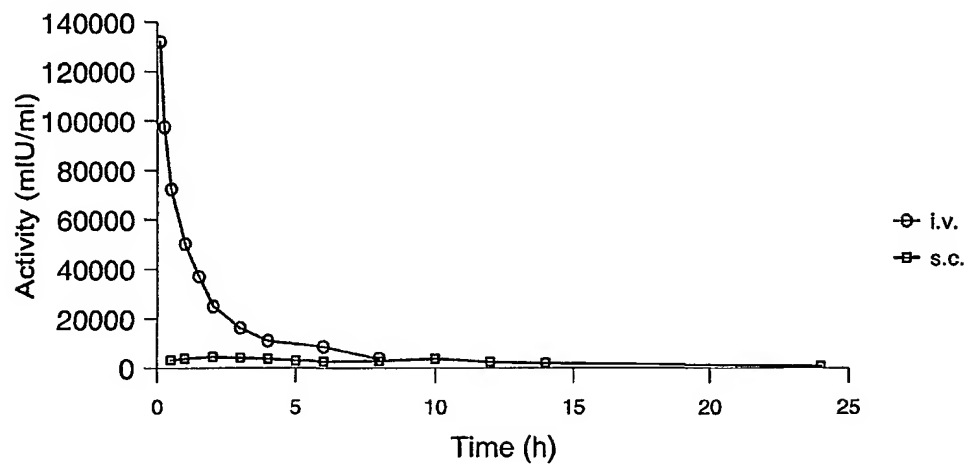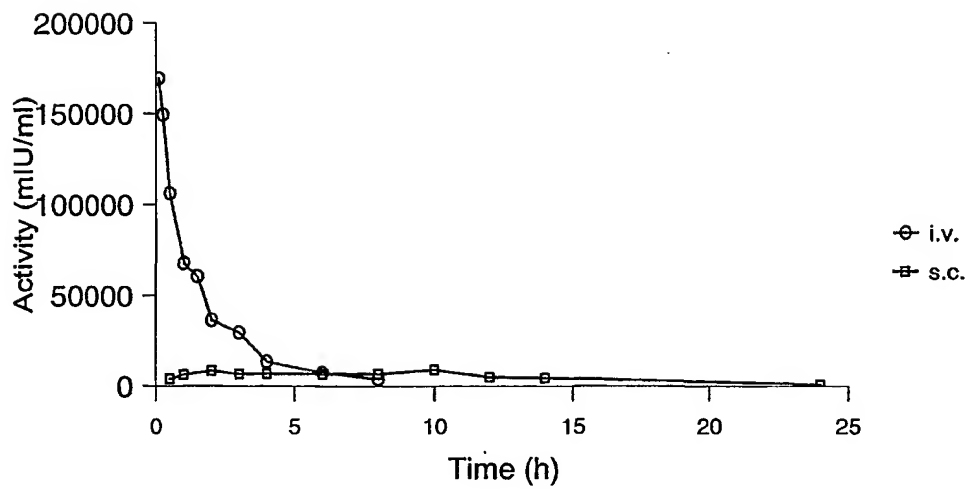
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Fig. 2a

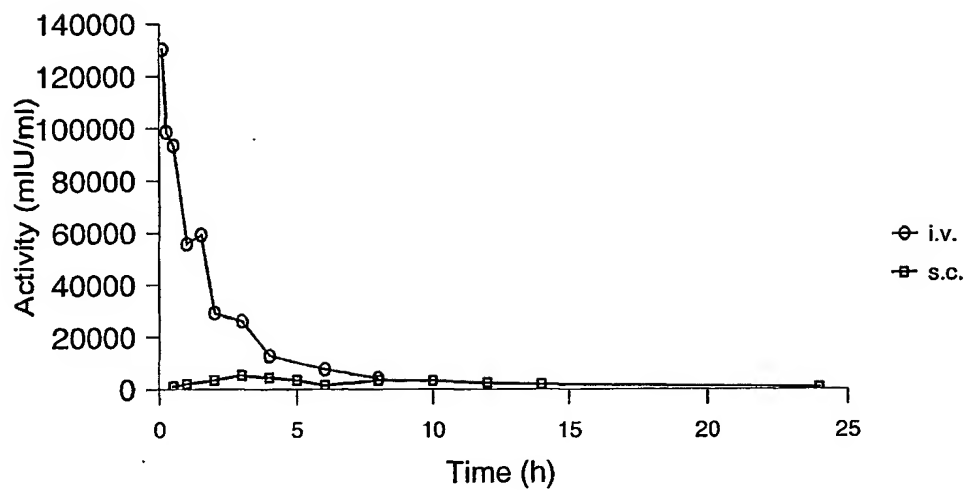Animal = 2

Fig. 2b

Animal = 3
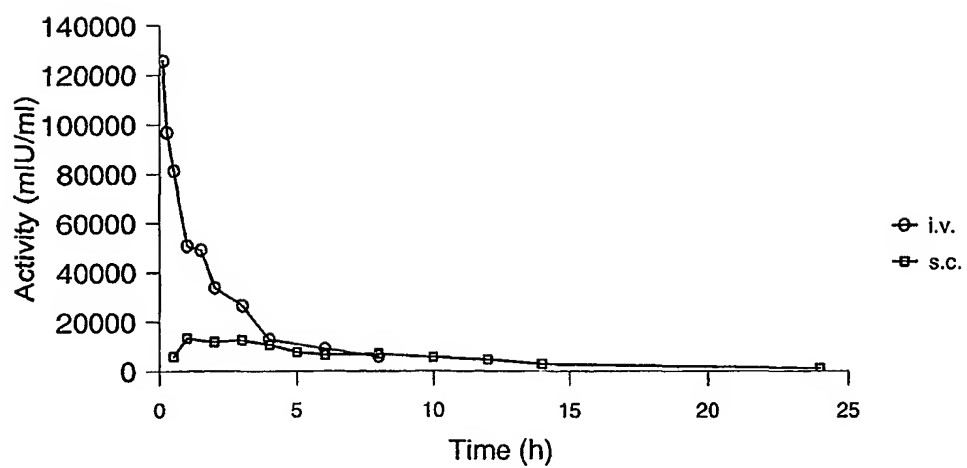

Fig. 2c

Animal = 4

Fig. 2d

FIG. 3

5

The amino acid sequence of native human coagulation Factor VII (wild-type Factor VII):

10   Ala-Asn-Ala-Phe-Leu-GLA-GLA-Leu-Arg-Pro-Gly-Ser-Leu-GLA-Arg-GLA-Cys-Lys-
                5   10   15

      GLA-GLA-Gln-Cys-Ser-Phe-GLA-GLA-Ala-Arg-GLA-Ile-Phe-Lys-Asp-Ala-GLA-Arg-
           20   25   30   35
15
      Thr-Lys-Leu-Phe-Trp-Ile-Ser-Tyr-Ser-Asp-Gly-Asp-Gln-Cys-Ala-Ser-Ser-Pro-
                40   45   50

      Cys-Gln-Asn-Gly-Gly-Ser-Cys-Lys-Asp-Gln-Leu-Gln-Ser-Tyr-Ile-Cys-Phe-Cys-
20   55   60   65   70

    • Leu-Pro-Ala-Phe-Glu-Gly-Arg-Asn-Cys-Glu-Thr-His-Lys-Asp-Asp-Gln-Leu-Ile-
                75   80   85   90

25   Cys-Val-Asn-Glu-Asn-Gly-Gly-Cys-Glu-Gln-Tyr-Cys-Ser-Asp-His-Thr-Gly-Thr-
                95   100   105

      Lys-Arg-Ser-Cys-Arg-Cys-His-Glu-Gly-Tyr-Ser-Leu-Leu-Ala-Asp-Gly-Val-Ser-
           110   115   120   125
30
      Cys-Thr-Pro-Thr-Val-Glu-Tyr-Pro-Cys-Gly-Lys-Ile-Pro-Ile-Leu-Glu-Lys-Arg-
                130   135   140

      Asn-Ala-Ser-Lys-Pro-Gln-Gly-Arg-Ile-Val-Gly-Gly-Lys-Val-Cys-Pro-Lys-Gly-
35   145   150   155   160

      Glu-Cys-Pro-Trp-Gln-Val-Leu-Leu-Leu-Val-Asn-Gly-Ala-Gln-Leu-Cys-Gly-Gly-
                165   170   175   180

40   Thr-Leu-Ile-Asn-Thr-Ile-Trp-Val-Val-Ser-Ala-Ala-His-Cys-Phe-Asp-Lys-Ile-
                185   190   195

      Lys-Asn-Trp-Arg-Asn-Leu-Ile-Ala-Val-Leu-Gly-Glu-His-Asp-Leu-Ser-Glu-His-
           200   205   210   ˙215
45

Asp-Gly-Asp-Glu-Gln-Ser-Arg-Arg-Val-Ala-Gln-Val-Ile-Ile-Pro-Ser-Thr-Tyr-
          220   225   230

5   Val-Pro-Gly-Thr-Thr-Asn-His-Asp-Ile-Ala-Leu-Leu-Arg-Leu-His-Gln-Pro-Val-
     235   240   245   250

     Val-Leu-Thr-Asp-His-Val-Val-Pro-Leu-Cys-Leu-Pro-Glu-Arg-Thr-Phe-Ser-Glu-
          255   260   265   270

10   Arg-Thr-Leu-Ala-Phe-Val-Arg-Phe-Ser-Leu-Val-Ser-Gly-Trp-Gly-Gln-Leu-Leu-
               275   280   285

     Asp-Arg-Gly-Ala-Thr-Ala-Leu-Glu-Leu-Met-Val-Leu-Asn-Val-Pro-Arg-Leu-Met-
          290   295   300   305

15
     Thr-Gln-Asp-Cys-Leu-Gln-Gln-Ser-Arg-Lys-Val-Gly-Asp-Ser-Pro-Asn-Ile-Thr-
               310   315   320

     Glu-Tyr-Met-Phe-Cys-Ala-Gly-Tyr-Ser-Asp-Gly-Ser-Lys-Asp-Ser-Cys-Lys-Gly-
20   325   330   335   340

     Asp-Ser-Gly-Gly-Pro-His-Ala-Thr-His-Tyr-Arg-Gly-Thr-Trp-Tyr-Leu-Thr-Gly-
          345   350   355   360

25   Ile-Val-Ser-Trp-Gly-Gln-Gly-Cys-Ala-Thr-Val-Gly-His-Phe-Gly-Val-Tyr-Thr-
               365   370   375

     Arg-Val-Ser-Gln-Tyr-Ile-Glu-Trp-Leu-Gln-Lys-Leu-Met-Arg-Ser-Glu-Pro-Arg-
          380   385   390   395

30
     Pro-Gly-Val-Leu-Leu-Arg-Ala-Pro-Phe-Pro
          400   405 406

35